Insights into plant extracellular ATP signalling revealed by the discovery of an ATP-regulated transcription factor

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In addition to its fundamental function as a universal cellular energy carrier, ATP has emerged as an extracellular signalling molecule with crucial roles in diverse aspects of plant growth and stress-adaptive responses (Tanaka *et al*., 2010). Genetic evidence identifying the first plant extracellular ATP (eATP) receptor in the plasma membrane (Choi *et al*., 2014) cemented eATP as an authentic signal for cell-cell communications. While the mechanisms of eATP perception and signalling are much clearer in animal cells, our understanding of equivalent mechanisms in plants is limited. Receptor activation triggers a rapid spike in cytosolic Ca^{2+} and biosynthesis of downstream messengers, such as reactive oxygen species, nitric oxide, and phosphatidic acid (Tanaka *et al*., 2010). Converting these early biochemical responses into specific physiological outputs requires a network of signal transduction and gene-regulatory proteins with key functions in altering gene expression. Identifying such proteins should provide useful insights into how eATP works in plants.

In this issue, Zhu *et al*. (2020) investigated the role of a stress-responsive transcription factor in mediating the effects of ATP on Arabidopsis roots. The Arabidopsis gene coding for Redox-Responsive Transcription Factor 1 (RRTF1) is rapidly stimulated (within minutes) on exposure to exogenous ATP. In addition to gene activation, ATP treatment also triggered translocation of recombinant RRTF1-YFP from the cytosol to the nucleus, where it presumably binds to an as yet unidentified DNA element in the promoter sequences of target genes. Chromatin immunoprecipitation and DNA sequencing were used to screen for genes that could be putative targets of RRTF1. Randomly selected candidates from this gene list were responsive to exogenous ATP. Importantly, the ATP-induced response of candidate genes seen in wildtype plants was abrogated in loss-of-function *rrtf1* mutants. Somewhat surprisingly, ATP-induced activation of *RRTF1* expression is not abolished in loss-of-function *dorn1* mutants lacking a functional copy of the eATP receptor DORN1. This suggests that multiple types of eATP receptors exist in plants, as is the case in animal cells (Di Virgilio and Adinolfi, 2017).

Early studies by Stanley Roux's group established the impact of exogenous ATP on auxin distribution and Arabidopsis root growth (Tang *et al*., 2003). ATP suppresses root elongation and disrupts gravitropic root growth, possibly a result of increased auxin retention and redistribution in root tips (Tang *et al*., 2003). When grown on ATP-free solid medium stacked above medium infused with ATP, Arabidopsis roots initially showed normal gravitropic growth, but on encountering ATP, they displayed strong avoidance growth behaviour characterised by loss of gravitropism (Liu *et al*., 2012). These physiological responses to ATP are thought to arise from altered distribution of auxin. Zhu *et al*. (2020) have now demonstrated that the ATP effects on auxin spatial profiles and root growth are mediated via RRTF1. ATP-induced spread of auxin distribution beyond the quiescent zone (to cortical and epidermal cells) seen in wildtype roots is abolished in loss-of-function *rrtf1* mutants. This coincides with blockade, in *rrtf1* mutants, of the ATP-induced proliferation of lateral roots and the inhibition of tap root growth phenotype that appears in wildtype plants. Furthermore, the characteristic acute bending response to ATP addition in dark-grown seedlings is averted in the *rrtf1* knockout mutants, supporting the notion that RRTF1 is a key regulatory protein in eATP-induced physiological processes.

Gary Stacey's group generated a fusion protein between a cellulose-binding domain peptide and luciferase for use in visualising eATP *in vivo*. They found that eATP is predominant in

regions of roots with active cell division and cell expansion (Kim *et al*., 2006), suggesting a key role for eATP in root growth. The results of Zhu *et al*. (2020) now provide at least some insight into how this eATP might contribute to this growth. Chromatin immunoprecipitation and DNA sequencing results point to a potential central regulatory role for RRTF1 in Arabidopsis growth processes and stress responses. Putative target genes identified in the analysis participate in diverse growth processes, including hormone metabolism or signalling and cell division. Transcription factors enriched for included lateral root formation, gravitropic growth control, and stress response. This implicates RRTF1 as a key node through which eATP regulates multiple processes controlling plant growth (Figure 1).

The fundamental question in this field is: why do plants use ATP, a major intracellular metabolite, as an extracellular signal? The hypothesis that eATP is a danger-warning signal associated with cell damage has been proposed (Tanaka *et al*., 2014), and it makes sense in situations where stress is associated with stress/damage-dependent intracellular ATP release. However, secretion of ATP in healthy and actively growing tissues (Kim *et al*., 2006) does not fit with such an interpretation. Identification of RRTF1 as an ATP-responsive factor with a suite of putative target genes involved in cell division, cell expansion, and gravitropic growth (Zhu *et al*., 2020) suggests that eATP has a fundamental function in growth processes that is above its role as a danger signal (Figure 1). Perhaps the link established by Zhu *et a*l. (2020) between RRTF1 and genes required for active growth might explain why an energy molecule (ATP) abundant in actively-growing sink tissues (such as root tips) is an appropriate switch to turn on this form of signalling. This leads to the second question: why does ATP need to signal from the apoplast instead of signalling from within the cell? Firstly, the apoplast represents a compartment that may function to demarcate the extracellular signalling ATP pool from the intracellular energy pool. Secondly, the recent hypothesis that apoplast signalling represents a mechanism for collective decision-making in similar fashion to quorum-sensing bacteria identified eATP signalling as a classic example (Chivasa and Goodman, 2019). This study (Zhu *et al*., 2020) has now raised the possibility of the existence of additional receptor protein(s), which will certainly be subject to future research in addressing these fundamental questions.

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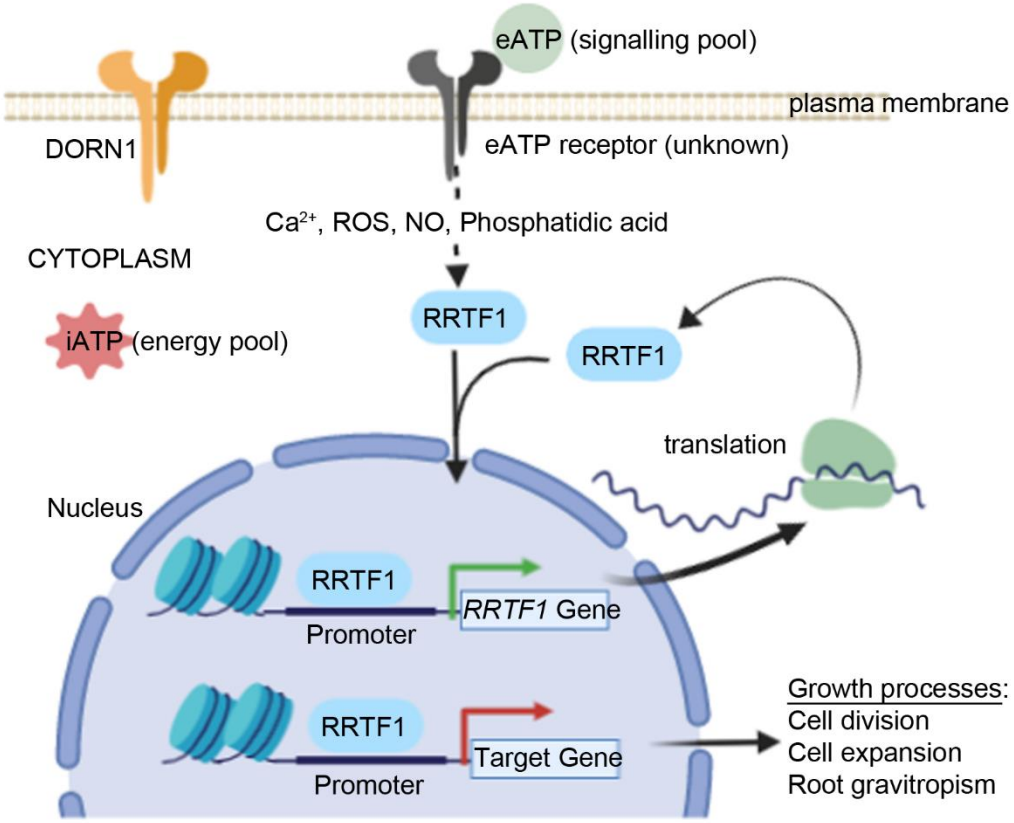

Figure 1. Schematic model of eATP signalling in root growth.
Extracellular ATP signals via an as yet unknown plasma membrane receptor (which is not DORN1) to activate second messengers, such as Ca^{2+} ions, reactive oxygen species (ROS), nitric oxide (NO), and phosphatidic acid. Downstream signalling leads to relocation of RRTF1 from the cytoplasm into the nucleus, where it binds to its own promoter as well as promoter sequences of target genes. Self-transcriptional activation serves as a signal amplification loop to ensure a rapid, sustained and robust reprogramming of the transcriptome. RRTF1-induced activation of target genes regulates growth processes, such as cell division, cell expansion, and root gravitropism. In this model, the plasma membrane demarcates the intracellular ATP (iATP) pool used for energy from the eATP pool used for signalling. Diagram based on the findings of Zhu *et al*. (2020).